# 19 Selenium

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**Key Points**

1. Evidence is accruing that the level of intake of selenium (Se) affects the risk of cancer and may even inhibit its spread from a primary tumour. Se exists in a number of dietary forms all of which are capable of being converted to hydrogen selenide ($H_2Se$), a crucial molecule in Se metabolism. The nutritional functions of Se are carried out by the selenoproteins which contain Se in the form of selenocysteine (Sec).
2. A number of parallel and/or consecutive mechanisms are likely to be involved in the anti-cancer effects of Se. Evidence exists for involvement of the selenoproteins, of methylated precursors that can generate methyl selenol and of redox-cycling superoxide and hydrogen peroxide generated by oxidation of hydrogen selenide, all of which have been associated with anti-cancer effects. Thus Se compounds can modify critical sulfhydryl groups to inhibit or promote tumor cell metabolism and cell transformation.
3. Selenoenzymes are involved in antioxidant protection and anti-inflammatory effects, and may enhance the cell-mediated immune response. Se compounds can cause cell cycle arrest and apoptosis, enhance DNA repair and reduce cancer cell migration. Importantly in relation to prostate cancer, Se can down-regulate the androgen receptor.
4. While there has been fairly general acceptance that a Se metabolite, methyl selenol, is a proximal anti-carcinogen at supra-nutritional doses, data linking cancer risk with the presence of selenoprotein polymorphisms and hypermethylation of promotor regions of selenoprotein genes has also implicated selenoproteins in anti-cancer effects. Numerous cohort and nested case–control studies have shown that higher Se status is associated with a lower risk of malignancies or death from cancer. However, such evidence is subject to some uncertainty owing to the effect of inflammation on plasma or serum Se concentration which can long precede the appearance of clinical symptoms.
5. Randomized clinical trials are not subject to such effects. Data from the Nutritional Prevention of Cancer randomized trial have shown a significant protective effect of supplementation with 200 μg Se/d, as high-Se yeast, on cancer incidence and mortality with the most notable effect being on prostate cancer, with lesser effects on colorectal and lung cancers. Significant effects were confined to males, were most pronounced in former smokers and in those with plasma Se < 105 μg Se/L at baseline, a level common in European populations. By contrast, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) that gave 200 μg Se/d, as selenomethionine, to men of replete Se status [serum Se 136 (range 123–150) μg /L] showed no benefit of Se supplementation over placebo. The need for further randomised trials in populations of low baseline Se status, in women, and with a lower Se dose, e.g. 100 μg Se/d, is argued. Trials with the methyl selenol precursor, *Se*-methylselenocysteine, also appear warranted as do trials in

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subjects of known selenoprotein SNP genotype, as we can no longer assume that each person's Se requirement to reduce cancer risk is the same.
6. In the meantime, there is no justification for increasing Se intake in persons with plasma Se above around 125 μg Se/L, though a case can certainly be made that an increased intake may benefit those whose plasma Se falls below 105 μg Se/L. However, definitive guidelines on optimal intake await further research and, for individuals rather than populations, these must ultimately be genotype related.

**Key Words:** Selenium; cancer; mechanism; selenomethyl-selenocysteine; single-nucleotide polymorphisms; epidemiology; hypermethylation; acute phase response

## 1. INTRODUCTION

Evidence is accruing that the level of intake of selenium (Se) affects the risk of cancer and may even inhibit its spread from a primary tumour. The nature of the Se species involved in anti-cancer processes and the extent to which the selenoproteins are relevant is still a matter of speculation and much ongoing experimental work.

Se is an unusual trace element in having its own codon in mRNA that specifies its insertion into selenoproteins as selenocysteine (Sec), by means of a mechanism requiring a large Sec-insertion complex. Unlike the other 20 amino acids, Sec is biosynthesised on its own tRNA, Sec $tRNA^{[Ser]Sec}$, from selenophosphate as the Se source. The insertion of Sec is specified by the UGA codon in mRNA. However, as UGA is also a stop codon, the presence of a stem-loop structure in mRNA – a SECIS (Sec Insertion Sequence) element – downstream from UGA in the 3′-mRNA-untranslated region, is also required for UGA to be read as selenocysteine. SECIS elements function by recruiting additional factors including the SECIS-binding protein, the Sec-specific elongation factor and Sec $tRNA^{[Ser]Sec}$ to form the large Sec-insertion complex required for the synthesis of selenoproteins and known as the selenosome *(1–3)*. This complex insertion machinery for selenoprotein production has implications for our Se requirements for cancer prevention. The human selenoproteome consists of 25 selenoproteins *(4)*.

Se exists in a number of dietary forms all of which are capable of being converted to hydrogen selenide ($H_2Se$), a central molecule in Se metabolism that can be further metabolised to selenoproteins, methylated or converted to selenosugars in the excretory pathway or oxidised to generate superoxide and hydrogen peroxide (Fig. 1) *(5)*. Selenomethionine (SeMet) is the main dietary form, particularly from grain or cereal sources. It can be metabolised to hydrogen selenide for conversion into selenoproteins but can also be incorporated indiscriminately into any body protein in place of methionine where it can remain until released by catabolism when it can undergo further metabolism. Selenocysteine is ingested in selenoproteins from animal sources and again must be metabolised to hydrogen selenide before further utilisation. Other food sources, notably plants from the *Allium* and *Brassica* families, contain *Se*-methylselenocysteine and γ-glutamyl-*Se*-methyl-selenocysteine which are readily converted to the potent anti-carcinogen, methylselenol ($CH_3SeH$), without the need for conversion to hydrogen selenide *(5)*. With regard to inorganic sources, a little selenate is present in vegetables *(5)* but selenite, though a component of some Se supplements and the form chosen for many human, animal and in vitro studies is basically absent from

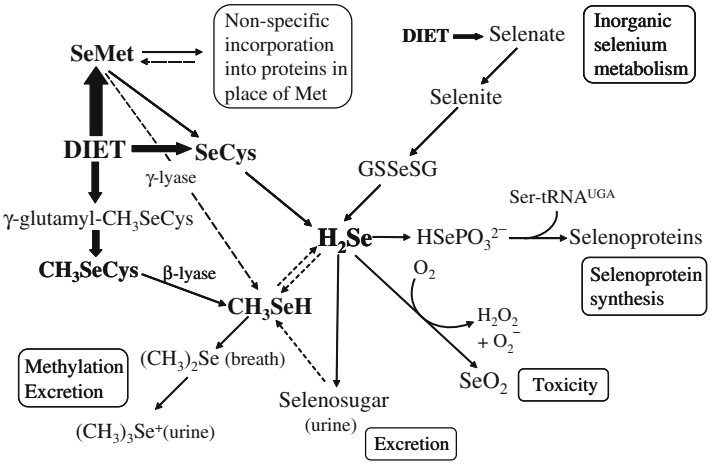


**Fig. 1.** Metabolic pathway of dietary Se in humans [Rayman et al. *(111)*] Abbreviations: SeMet, selenomethionine; SeCys, selenocysteine; γ-glutamyl-$CH_3$SeCys, γ-glutamyl-*Se*-methyl-selenocysteine; $CH_3$SeCys, *Se*-methyl-selenocysteine; $H_2Se$, hydrogen selenide; $CH_3SeH$, methyl selenol; $(CH_3)_2Se$, dimethyl selenol; $(CH_3)_3Se^+$, trimethyl selenonium ion; GSSeSG, selenodiglutathione; $HSePO_3^{2-}$, selenophosphate; $SeO_2$, selenium dioxide.

the food chain. Synthetic Se compounds such as methyl seleninic acid that readily yield methylselenol in vitro have been developed for use in model systems.

## 2. RATIONALE FOR AN EFFECT OF SELENIUM ON CANCER PREVENTION AND TREATMENT

A number of parallel and/or consecutive mechanisms are likely to be involved in the anti-cancer effects of Se. Evidence exists for involvement of the selenoproteins, of methylated precursors that can generate methyl selenol and of redox-cycling superoxide and hydrogen peroxide generated by oxidation of hydrogen selenide, all of which have anti-cancer effects. The anti-carcinogenic mechanisms by which Se acts have previously been reviewed *(6–10)*. Anti-cancer mechanisms are summarised in Fig. 2 and further discussed below.

### *2.1. Modification of Critical Sulfhydryl Groups*

Se can act very generally through its redox-active compounds (e.g. methylated Se metabolites) which can affect cellular proteins by modification of critical cysteine residues, particularly when clustered *(6, 11)*. This may in turn have downstream effects on signal transduction and gene transcription *(12)*. Se adducts of the selenotrisulfide (S–Se–S) or selenenylsulfide (S–Se) type may form while disulfide bonds may be made or broken. Ganther has proposed that Se catalysis of reversible cysteine/disulfide transformations that occur in a number of redox-regulated proteins, [e.g. p53 *(13)*, protein kinase C (PKC) *(14)*] including transcription factors, may be a chemopreventive mechanism *(11)*. For instance, PKC regulates tumor promotion and cell growth by inducing

- Modification of critical sulfhydryl groups to inhibit (e.g. protein kinase C, NF-κB, AP-1) or promote (p53) redox-sensitive factors that impair tumor cell metabolism and cell transformation
- Antioxidant protection (selenoenzymes/selenoproteins)
- Anti-inflammatory effect (partly *via* selenoenzymes/selenoproteins e.g. SEPS1)
- Enhancement of cell-mediated immune response
- Maintenance of genome stability – prevention of DNA damage/enhancement of DNA repair
- Cell cycle arrest – decreases cell proliferation
  - S/G2 cell cycle arrest ($H_2Se$)
  - G1 cell cycle arrest (methyl selenol)
- Apoptosis (necrosis)
  - caspase-mediated (methyl selenol)
  - genotoxic-mediated ($H_2Se$)
  - ER-stress-mediated
- Reduced tumor cell invasion/migration capacity (both selenite and methyl selenol)
- Inhibition of angiogenesis: effect on gene expression of MMPs and TIMPs (methyl selenol and $H_2Se$)
- Activation of p53 tumor-suppressive activity
- Inactivation of protein kinase C
- Upregulation of phase II carcinogen-detoxifying enzymes
- Androgen receptor down-regulation (relevant to prostate cancer)

**Fig. 2.** Anti-carcinogenic mechanisms of Se (see text for details and references).

activation of transcription factors and by increasing the expression of key enzymes, such as ornithine decarboxylase, inducible nitric oxide synthase and cyclooxygenase-2 *(14)*. Thus inactivation of PKC by redox-active selenometabolites can inhibit tumor promotion, cell growth, invasion and metastasis and promote the induction of apoptosis *(14)*.

A further example of the effect of redox modification of thiol/disulfide bonds is that it results in protein misfolding or unfolding *(15)*. Newly synthesized proteins are particularly vulnerable before they are properly folded in the endoplasmic reticulum (ER). Thus, it is highly plausible that Se metabolites can produce ER stress, which if too severe, exceeding the capacity for repair, will trigger the signal for apoptosis *(15)*. It appears that low doses of Se (e.g. as methylseleninic acid, a methyl selenol precursor) preferentially activate the rescue arm of the ER stress response [likely involving

selenoprotein S, SEPS1 *(16)*, whereas high doses lead to the assembly of the apoptotic machinery. Se may have a dichotomous effect such that it favours survival response in normal cells but facilitates the apoptotic response in cancer cells *(15)*.

### *2.2. Antioxidant Protection (Selenoenzymes/Selenoproteins)*

Se, as selenoenzymes reduces oxidative stress. Selenoenzymes can reduce hydrogen peroxide and lipid hydroperoxide intermediates in the cyclooxygenase and lipoxygenase pathways preventing further conversion to reactive oxygen species (ROS) that can cause oxidative stress, damaging DNA and other macromolecules and promoting cancer *(17)*.

That the ability of Se in selenoproteins to reduce oxidative stress is relevant to its anti-cancer effects is suggested by the modification of these effects by other antioxidant nutrients. Thus the strongest effect of Se on cancer risk has been shown among those with the lowest levels of dietary antioxidant vitamins and carotenoids *(18–24)* and particularly at low α-tocopherol concentrations *(25, 26)*. Smoking modifies the effect of Se on cancer risk, demonstrating the operation of an antioxidant mechanism *(27, 28)*.

A further indication of a link between the antioxidant capacity of Se and cancer risk is seen in the modification of that Se-dependent risk by a polymorphism in manganese superoxide dismutase (SOD2), the primary antioxidant enzyme in mitochondria. SOD2 has a polymorphism (Val16Ala, rs4880) that has been shown to alter the secondary structure of the mitochondrial import sequence of the superoxide dismutase protein such that the Ala16 variant is imported more efficiently into the mitochondrial matrix, resulting in higher enzyme activity *(29)*. Men carrying the Ala allele are therefore likely to produce more hydrogen peroxide which promotes prostate cancer cell proliferation and migration and induces matrix metalloproteinases required for tumour invasion *(30–32)*. There is an interaction between Se status and this polymorphism owing to the requirement for a selenoenzyme, GPx, to remove the hydrogen peroxide (there being no catalase in mitochondria). Thus men in the bottom quartile of Se status who were SOD2 Ala homozygotes had a significantly higher risk of aggressive prostate cancer than Val/Ala or Val/Val men (1.89; 95% CI 1.01, 3.56) but if they were in the top quartile, their risk was significantly lower, presumably because they could both efficiently remove superoxide *and* make enough GPx to deal with the extra hydrogen peroxide formed *(33)*. The interdependence of SOD2, Se status and prostate cancer risk implies a role for the antioxidant selenoenzymes.

### *2.3. Anti-inflammatory Effect (Partly via Selenoenzymes/Selenoproteins)*

Inflammation is known to promote tumour growth *(34)*. Macrophage activation is a crucial step in the inflammatory process that forms the underlying basis of cancer progression *(35)*.

Se reduces inflammation by a number of mechanisms in at least some of which selenoproteins are known to be involved (Fig. 3). Se aids in the shunting of arachidonic acid towards endogenous anti-inflammatory mediators as an adaptive response to protect cells against pro-inflammatory gene expression induced by oxidative stress. Thus Se supplementation in macrophages increases the production of 15d-$PGJ_2$ (by the COX-1 pathway), an endogenous inhibitor of a key kinase of the NF-κB cascade, IκB-kinase

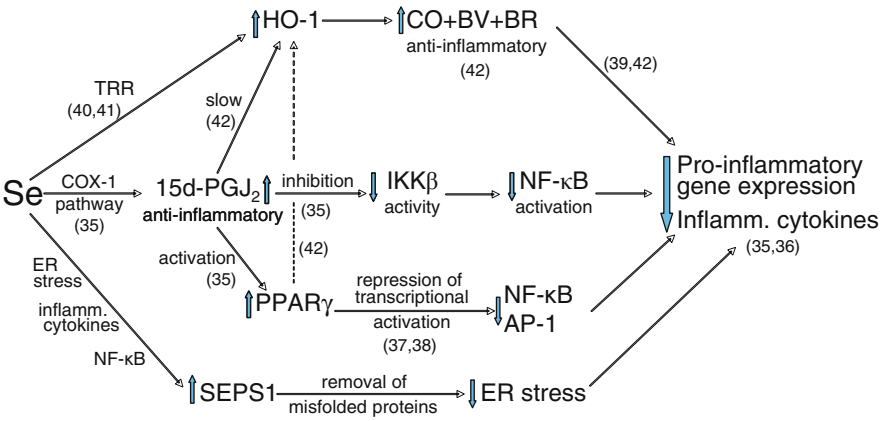


**Fig. 3.** Some mechanisms by which Se reduces inflammation resulting from oxidative stress *(39–42)*. Abbreviations: ER, endoplasmic reticulum; SEPS1, selenoprotein S; 15d-PGJ2, 15-deoxy-Δ12, 14-prostaglandin J2; IKKβ, IκB-kinase β; TRR, thioredoxin reductase; BV, billiverdin; BR, billirubin.

β (IKKβ) *(36)*. This effect results in decreased activation of NF-κB and down-regulates expression of inflammatory genes such as COX-2, TNF-α, IL-6 and VCAM-1 *(36, 37)*. In a second Se-dependent anti-inflammatory mechanism acting through 15d-$PGJ_2$, Se-supplemented macrophages activate the peroxisome proliferator-activated nuclear receptor-γ (PPAR-γ) *(36)*, repressing inflammatory gene expression *(38, 39)*.

HO-1 is an inducible enzyme that is upregulated in oxidative stress with cytoprotective and anti-inflammatory functions linked to its removal of the pro-oxidant, heme, and its production of the antioxidant bilirubin and the vasodilatory, anti-inflammatory carbon monoxide (CO) *(40)*. Se has been shown to upregulate HO-1 by a number of pathways resulting in reduced expression of pro-inflammatory genes *(41, 42)* (see Fig. 3) *(43, 44)*.

Selenoprotein S (SEPS1) is an endoplasmic reticulum (ER) membrane protein involved in the control of inflammation and genetic variation in selenoprotein S has been shown to influence the inflammatory response *(45, 46)*. By regulating cellular redox balance, SEPS1 protects the ER against the deleterious effects of oxidative stress. It has a role in the removal of stressor-induced misfolded proteins from the ER, preventing the accumulation of these proteins and the subsequent stress response that leads to activation of NF-κB, pro-inflammatory cytokine gene transcription and the inflammation cascade. Impairment of SEPS1 is directly associated with increased cellular cytokine production and release. There appears to be a regulatory loop whereby cytokines stimulate the expression of SEPS1 which in turn suppresses cytokine production *(45)*.

### *2.4. Enhancement of Cell-Mediated Immune Response*

Cytotoxic lymphocytes and natural killer cells are able to destroy tumour cells. We know that Se is important to immune regulation but there is little understanding of how it acts at the molecular level. A recent study has shed some light on this process. Mice with selenoproteinless T cells were generated by cell type-specific ablation of the Sec

tRNA([Ser]Sec) gene (*Trsp*) *(47)*. The resultant selenoprotein deficiency caused defects in the development of functionally mature T cells and their T-cell-receptor-dependent activation. Furthermore, selenoprotein deficiency led to oxidant hyperproduction in T cells, suppressing T cell proliferation in response to T-cell-receptor stimulation. Selenoprotein expression in T cells appears to be crucial for their ability to proliferate in response to T-cell receptor stimulation.

Se supplementation (as sodium selenite) enhanced the immune response of healthy volunteers and cancer patients *(48, 49)*. Thus supplementation of healthy volunteers with a "selenium replete" status with 200 μg/d Se for 8 wk increased the ability of human peripheral blood lymphocytes to respond to stimulation with alloantigen *(48)*. The supplementation regimen resulted in 118% increase in cytotoxic lymphocyte-mediated tumor cytotoxicity and 82.3% increase in natural killer cell activity as compared to baseline values. The effect appeared to be related to the ability of Se to enhance the expression of receptors for the growth regulatory lymphokine interleukin-2, and consequently, the rate of cell proliferation and differentiation into cytotoxic cells. Patients supplemented with 200 μg/d Se during therapy for squamous cell carcinoma of the head and neck, e.g. surgery, radiation or surgery and radiation, had a significantly enhanced cell-mediated immune response in contrast to patients in the placebo arm of the study who showed a decline in immune responsiveness during therapy *(50)*.

### *2.5. Maintenance of Genome Stability – Prevention of DNA Damage/Induction of DNA Repair*

Se (presumably as selenoenzymes) can prevent damage to DNA that causes single- and double-strand breaks. Thus Se (as sodium selenite) and overexpression of GPx1 protected mammalian cells against UV-induced DNA damage *(51)*. Protection appeared to be dependent on functional activity of BRCA1, a protein involved in maintaining the integrity of the human genome which helps to repair DNA double-strand breaks. In a further set of studies, Se, as SeMet, protected normal fibroblasts from subsequent DNA damage by selective induction of the DNA repair branch of the p53 pathway involving interaction with BRCA1 and Redox-factor 1 (Ref1) *(52)*.

Given the requirement for functional BRCA1 activity found in the studies described above, it is perhaps surprising that Se appears to protect women born with a mutation in *BRCA1* that presumably leads to an abnormal BRCA1 protein. These women carry a lifetime risk of breast cancer of 80% and a lifetime risk of ovarian cancer of 40% *(53)*. When blood lymphocytes from *BRCA1* carriers are exposed to bleomycin, a known mutagen that induces double-strand breaks, an increased frequency of chromosome breaks per cell occurs, i.e. 0.58 in *BRCA1* carriers *vs*. 0.39 in non-carriers *(53)*. In 32 female *BRCA1* carriers supplemented with Se (276 μg/d as sodium selenite) for 1–3 months, the frequency of chromosome breaks per cell was significantly reduced from 0.63 before supplementation with Se to 0.40 after supplementation with Se, bringing it to the level in non-carrier controls. Thus Se may have the potential to reduce breast cancer risk in these women.

The effect of Se status on protection from DNA damage was investigated in New Zealand men, aged 50–75 year, judged to be at risk of prostate cancer (PSA >

4). The comet assay, carried out in blood leukocytes from those with serum Se below the mean, showed a significant inverse relationship with overall accumulated DNA damage ($p = 0.02$) *(54)*. As mean serum Se was measured as 98 ± 17 μg/L, this suggests that serum levels above 98 μg/L are required for the prevention of DNA damage in New Zealand men.

### *2.6. Cell Cycle Arrest – Decreases Cell Proliferation*

Se compounds have been shown to cause a block in progression of the cell cycle. The resulting inhibition of growth may allow DNA repair to take place. In the case of selenite, the mechanism probably involves interaction with glutathione resulting in conversion to selenide and then oxidative metabolism to superoxide and hydrogen peroxide which can cause DNA strand breaks, triggering S-phase/G2 cycle arrest *(55–58)*. By contrast, methyl selenol precursors can induce G1 cell cycle arrest without single-strand breaks and with or without caspase induction and p53 involvement *(8*, *56–58)*.

### *2.7. Apoptosis (Necrosis)*

One of the most significant mechanisms by which Se reduces cancer risk is by the induction of apoptosis in cancer cells. In the case of selenite, in vitro experiments reveal a genotoxic mechanism involving superoxide and hydrogen peroxide which cause DNA single-strand breaks culminating apparently in necrosis or apoptosis, depending on the study *(57*, *59)*. The caspases are not involved *(6)*. By contrast, methylated forms of Se (*Se*-methylselenocysteine, methylseleninic acid or methylselenocyanate) may not cause DNA strand breaks and induce apoptosis apparently by activating key enzymes in the caspase pathway *(8*, *55*, *57*, *59*, *60)*.

A further mechanism by which methylated Se can induce apoptosis is by the creation of endoplasmic reticulum (ER) stress. While low doses of Se preferentially activate the rescue arm of the ER stress response, high doses lead to the assembly of the apoptotic machinery. Se appears to have a dichotomous effect: it favours survival response in normal cells and facilitates apoptotic response in cancer cells *(15)*.

### *2.8. Reduced Tumor Cell Migration and Invasion*

Cancer cell invasion requires coordinated processes, such as changes in cell–cell and cell–matrix adhesion, degradation of the extracellular matrix and cell migration *(61)*. Se, in a number of forms, inhibits the invasion of tumor cells, reducing the risk of metastasis:

- Dietary supplementation of selenomethionine reduced experimental metastasis of melanoma cells in mice and inhibited the growth of metastatic tumours that formed in the lungs *(62)*.
- Dietary supplementation of selenite, at 2- and 4-ppm, reduced pulmonary metastasis of B16BL6 melanoma cells in C57BL/6 mice and inhibited the growth of the metastatic tumours in the lungs *(63)*.
- Selenite inhibited invasion of HT1080 human fibrosarcoma cells and adhesion of the cells to the collagen matrix *(61)*.

– In HT1080 human fibrosarcoma cells, exposure to sub-micromolar concentrations of methylselenol (from seleno-L-methionine and methioninase) inhibited the migration and invasion rate of the tumor cells by up to 53 and 76%, respectively, when compared with the control tumor cells *(64)*.

### *2.9. Inhibition of Angiogenesis*

Blood vessel formation, or angiogenesis, is required for the growth and metastasis of tumours. Two proteins critical for angiogenesis are matrix metalloproteinase-2 (MMP-2) produced by vascular endothelial cells, which degrades the extracellular matrix, and vascular endothelial growth factor (VEGF) produced by cancer epithelial cells *(65*, *66)*.

Thus, in the rat mammary carcinoma model system, increased Se intake (as Se-enriched garlic, sodium selenite or *Se*-methylselenocysteine) significantly reduced intratumoral microvessel density and inhibited the expression of vascular endothelial growth factor (VEGF) *(66)*. By contrast, treatment caused no change in microvessel density of the uninvolved mammary glands.

In vitro data also show that both selenite and monomethyl Se inhibit the invasion of tumor cells by their effect on endothelial matrix metalloproteinase (MMP) and/or VEGF expression:

– Selenite inhibited the invasion of HT1080 human fibrosarcoma cells, adhesion of cells to the collagen matrix and reduced the expression of MMP-2 and -9 and urokinase-type plasminogen activator, which are involved in matrix degradation, while increasing the expression of a tissue inhibitor of metalloproteinase-1 (TIMP-1) *(61)*.
– Short-term exposure of human umbilical vein endothelial cells (HUVECs) to the methylselenol precursors, methylseleninic acid (MSeA) and methylselenocyanate (MSeCN) decreased the MMP-2 gelatinolytic activity in a concentration-dependent manner largely through a decrease of the MMP-2 protein level *(65)*.
– Exposure of human prostate cancer (DU145) and breast cancer (MCF-7 and MDA-MB-468) cell lines to MSeA, but not to selenite, led to a rapid and sustained decrease of cellular and secreted VEGF protein levels *(65)*. The concentration of monomethyl Se required for inhibiting endothelial expression of MMP-2 and cancer epithelial expression of VEGF was within the physiological range and much lower than that needed for apoptosis induction *(67)*.
– Sub-micromolar methylselenol (from seleno-L-methionine and methioninase) increased not only the enzyme activity of pro-MMP-2 (the active form of MMP-2) but also protein levels of anti-metastasic tissue inhibitor metalloproteinase (TIMP)-1 and TIMP-2 in HT1080 human fibrosarcoma cells giving a net effect of inhibition of pro-MMP-2 activation and carcinogenic potential *(64)*.

### *2.10. Activation of p53 Tumor-Suppressive Activity*

p53 is a transcription factor that activates a number of downstream genes that function in cellular responses to DNA damage. p53 activation is common to Se compounds but specific mechanisms differ between Se chemical forms *(68)*. SeMet activates the DNA

repair branch of the p53 pathway by redox regulation of key p53 cysteine residues *(13)*, while methyl seleninic acid and sodium selenite affect p53 phosphorylation in treated cells. Different Se chemical forms may differentially modify p53 for DNA repair or apoptosis in conjunction with a given level of endogenous or exogenous DNA damage *(68)*. Thus Se-enriched broccoli which is a rich source of *Se*-methyl-selenocysteine upregulates p53 and promotes apoptosis in Min mice *(69)*. There is some evidence that increase in p53 activity could also help to switch off angiogenesis in early lesions *(6)*.

### *2.11. Upregulation of Phase II Carcinogen-Detoxifying Enzymes*

Sources of some Se compounds [e.g. Se-enriched garlic, mushrooms, selenite, selenate, 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC)] have been shown to detoxify carcinogens and/or reduce DNA-adduct formation in rats and mice *(8)*. For example, prior feeding with a Se-garlic-containing diet (at 3 ppm Se, a source of *Se*-methylselenocysteine) for two weeks resulted in an elevation of glutathione *S*-transferase and uridine 5′-diphosphate-glucuronyltransferase activities to a maximum of 2- to 2.5-fold in liver and kidney *(70)*. There was a consistent reduction of all dimethylbenz[a]anthracene (DMBA) adducts in liver and mammary gland accompanied by a 40% increase in urinary excretion of DMBA metabolites over a 2-day period. These results are supported by microarray analysis that has shown that Se can upregulate genes related to phase II detoxification enzymes *(71)*.

### *2.12. Inactivation of PKC*

PKC is a signalling receptor that plays a crucial role in tumour promotion by oxidants that can be inactivated by redox-active selenometabolites (see above) *(14)*. Thus Se-induced inactivation of PKC may, at least in part, be responsible for the Se-induced inhibition of tumor promotion, cell growth, invasion, metastasis and for the induction of apoptosis *(14)*. *Se*-methylselenocysteine, an effective chemopreventive agent against mammary cell growth in vivo and in vitro (mouse mammary epithelial tumor cell line), was shown to decrease PKC activity *(72)*. Thus PKC may be an upstream target for *Se*-methylselenocysteine that may trigger downstream events such as the decrease in cdk2 kinase activity and DNA synthesis, elevation of *gadd* gene expression and finally apoptosis *(72)*.

### *2.13. Androgen Receptor Down-regulation (Relevant to Prostate Cancer)*

The prostate seems to be particularly sensitive to the anti-cancer effects of Se. The androgen receptor (AR) is a key mediator of prostate cancer progression. Androgen binding to the AR stimulates its translocation to the nucleus where it interacts with specific androgen-responsive elements (ARE) on the promoters of target genes. The interaction leads to the activation or repression of genes involved in the proliferation and differentiation of the prostate cells *(73)*. The inhibitory effect of Se on prostate cancer progression may be mediated through androgen receptor down-regulation *(73–75)*.

Se compounds have been shown to inhibit cell growth and induce apoptosis in both androgen-dependent and androgen-independent prostate cancer cells *(12)*. According to

Combs and Lü, sub-apoptotic concentrations of methylated Se reduce androgen receptor protein expression, inhibit androgen-stimulated PSA promoter transcription, reduce PSA expression and secretion and cause rapid PSA degradation *(76)*. Thus methylseleninic acid decreased the expression of androgen receptor and PSA in five human prostate cell lines *(73–75)*. Furthermore, methylseleninic acid inhibited the expression of a number of androgen-receptor-regulated genes that are consistently over-expressed in prostate cancer *(73)*: PSA, KLK2, ATP-binding cassette C4 (ABCC4, also known as MRP4), 24-dehydrocholesterol reductase (DHCR24, also known as seladin-1) and soluble guanylate cyclase 1 α3 (GUCY1A3).

Selenite has also been shown to inhibit AR expression and activity in LAPC-4 and LNCaP prostate cancer cells though by a different mechanism. Sp1 is a ubiquitously expressed transcription factor: its binding sequence is the major positive regulatory element in the AR promoter. Prostate cancer cells exposed to selenite had decreased Sp1 activity and reduced Sp1 expression in the nucleus whereas methylseleninic acid had no effect. The effect of selenite on Sp1 expression leading to inhibition of AR expression and activity was redox dependent, involving GSH and superoxide *(12)*.

## *2.14. Species of Se Responsible for Anti-cancer Effects*

There has been fairly general acceptance that a Se metabolite, methyl selenol, is a proximal anti-carcinogen at supra-nutritional doses, despite the fact that the presence of methyl selenol is only inferred from reactions of its precursors, most notably the model compound, methylseleninic acid *(77)*. Se doses large enough to support high, steady-state concentrations of methyl selenol are likely to be required.

Despite the fact that selenoproteins can reduce oxidative stress and inflammation and limit DNA damage, all of which have been linked to cancer risk, it was at first thought that selenoenzymes were not involved in anti-cancer mechanisms. This was largely because their activity/concentration was already believed to be optimized in the US population that showed reduced cancer risk on supplementation with 200 μg Se/d in the Nutritional Prevention of Cancer Trial *(25)*. However, it has recently become clear that optimal expression of selenoprotein P, the carrier of Se in the plasma, requires a higher intake, as yet undetermined, of dietary Se than other selenoproteins *(78)*. Furthermore, a substantial number of individuals may have a higher than average requirement for Se for efficient selenoprotein synthesis. Effects of functional polymorphisms in selenoprotein genes and of hypermethylation of their promoter regions have shown that the selenoproteins/selenoenzymes do appear to affect cancer risk, particularly at nutritional levels of intake.

### 2.14.1. Polymorphisms in Selenoproteins/Selenoenzymes Show an Effect on Cancer Risk

People differ substantially in their ability to increase selenoprotein activity in response to additional dietary Se *(79)*. This inter-individual variation in selenoprotein expression levels may be accounted for by SNPs in selenoprotein genes that determine the efficiency with which individuals can incorporate selenium into selenoproteins *(80–83)*. Thus requirements for dietary selenium for optimal protection against cancer

may be much higher in individuals carrying particular functional selenoprotein SNPs such as those described below in various selenoproteins.

### 2.14.2. Cytosolic Glutathione Peroxidase, GPx1

Some recent studies have reported a link between cancer risk and polymorphisms in the cytosolic glutathione peroxidase selenoprotein (*GPx1*) gene at Pro198Leu (rs1050450). Such a link might be explained by a genotype effect on enzyme activity. GPx1 with the Leu-allele has been reported to be less responsive to stimulation of its enzyme activity by selenium supplementation than GPx1 with the Pro-allele *(81)*. There have also been reports of a difference in GPx activity between the genotypes, e.g. in Danish women, the catalytic activity of GPx1 was lowered 5% for each additional copy of the variant Leu-allele ($p = 0.0003$) *(84)* while in a Californian study, male Leu/Leu homozygotes had significantly lower GPx1 activity than other genotypes *(85)*. However, that same Californian study found no difference in GPx activity by genotype in women. By contrast, two much smaller studies, one in a Finnish/Swedish population and one in men from the Former Yugoslav Republic of Macedonia (FYROM) could not detect a difference in erythrocyte GPx activity between GPx1 genotypes *(86*, *87)*. These disparities might be explained if the change in GPx activity were to be caused by another polymorphism that co-segregates with the studied polymorphism. There are several such candidate polymorphisms in GPx (http://egp.gs.washington.edu/directory.html) *(84)*.

*Lung:* Four studies have looked at the association between the Pro198Leu polymorphism and the risk of lung cancer *(83*, *88–90)*. Compared to Pro homozygotes, two studies – in Finland and Korea – found a significantly increased risk of lung cancer in Leu hetero-/homozygotes *(83*, *88)*, while a small US study found a significantly increased risk in never smoker Leu hetero-/homozygotes (only 13 cases), though a significantly decreased risk was found in elderly smokers *(89)*. The fourth study, carried out in Denmark, found that Leu/Leu homozygosity was associated with decreased risk *(90)*. The authors of the Danish study were themselves surprised by their result since they had previously shown that the variant Leu-allele was associated with a significant, although moderate, 5% lower erythrocyte GPx1 enzyme activity per allele *(84)*. They have suggested that the apparently protective effect of the Leu-allele of the GPx1 polymorphism may be caused by a co-segregating functional polymorphism in another gene in the same region of the genome and not by the GPx1 polymorphism *per se (90)*.

*Breast:* Five studies have investigated the effect of the Pro198Leu polymorphism on breast cancer risk with varying results. In a nested Danish case–control study of 377 cases and 377 controls, carriers of the variant Leu-allele had a 1.43 (95% CI 1.07–1.92) times higher risk of breast cancer compared with non-carriers *(84)*. Furthermore, the Leu/Leu genotype was found to be almost twice as common in DNA from breast cancer tissue from a tissue bank at the University of Illinois as in DNA from cancer-free individuals, while the Pro/Leu genotype was underrepresented, indicating loss of heterozygosity at this locus in breast tumour development *(81)*. The authors suggest that this may implicate GPx1 in the risk and development of breast tumours.

By contrast, in a Canadian case–control study of 399 cases of incident, invasive breast cancer and 372 controls, no association between breast cancer and GPx1 Pro198Leu was found *(91)*. Similarly, there was no evidence that the variant GPx1 genotype was associated with an increased risk of breast cancer in the Long Island Breast Cancer Study Project of 1,038 cases and 1,088 controls, except in nulliparous Leu homozygotes who had increased risk (OR 2.12, 95% CI 1.01–4.48) compared with parous Pro/Pro women *(92)*. Interestingly, though no association was observed between the polymorphism and breast cancer risk in the prospective Nurses' Health Study, where 1,323 women with breast cancer were compared with 1,910 controls *(93)*, an increased risk of breast cancer (OR 1.87, 95% CI 1.09–3.19) *was* observed in Leu homozygotes who were also homozygous for the Ala16 genotype of SOD2 (Val16Ala, as discussed above) *(94)*.

*Bladder:* Possession of the GPx1 Leu198 allele appears to confer an increased risk of bladder cancer and that risk is further raised in men that have one or two Ala alleles of the Val9Ala (more often described as Val16Ala, rs4880) MnSOD polymorphism *(95)*. In the 213 bladder cancer patients, the Pro/Leu genotype was significantly associated with advanced tumour stage: OR 2.58 (95% CI 1.07, 6.18, $p = 0.034$) for tumour stage T2–4 *vs*. Ta+1 when compared with the Pro/Pro genotype *(95)*.

*Prostate:* In the context of the above results, it is perhaps surprising that an overall protective effect of the variant GPx1 Leu-allele was found on prostate cancer risk in 82 prostate cancer cases and 123 control individuals in FYROM *(87)*. It is, however, somewhat suspicious that while heterozygous carriers of the variant Leu-allele had a significantly lower risk of prostate cancer compared with Pro homozygotes (OR 0.38, 95% CI 0.20–0.75, $p = 0.004$), Leu homozygotes had a non-significant and lesser reduction in risk. No significant differences in erythrocyte GPx activity by genotype were found in the healthy control group of 90 subjects.

*Other cancers*: No associations were found between the GPx Pro198Leu polymorphism and risk of basal-cell carcinoma or colorectal adenomas or carcinomas *(96, 97)*. However, loss of heterozygosity at GPx1 was found in a significant percentage of colorectal cancers (42%) *(98)* suggesting that loss of heterozygosity at the GPx1 locus is a common event in the development of colorectal cancer and that GPx1 or other tightly linked genes may be involved in the aetiology of this disease. Similarly DNA samples from head and neck tumours exhibited fewer heterozygotes and an increased frequency of the Leu/Leu genotype compared with DNA from the cancer-free population *(99)*.

### 2.14.3. 15 kDa Selenoprotein, Sep15

The 15 kDa selenoprotein (Sep15) is expressed at high levels in normal liver and prostate but at reduced levels in the corresponding malignant organs *(100)*. The *Sep15* gene lies on chromosome 1p22.3 at a locus commonly deleted or mutated in human cancers (*4, 82)* giving rise to expectations that this selenoprotein might be important to cancer risk. Two SNPs at positions 811 (C/T) and 1,125 (G/A) that are in strong allelic association have been studied in the 3′-UTR of the *Sep15* gene: G/A1125 lies within a functional SECIS element *(82)*. The T811-A1125 variant was more effective in supporting UGA readthrough than the C811-G1125 variant, but was less responsive to the addition of Se to the culture medium *(80, 101)*. Individuals possessing one or

other of these haplotypes may therefore differ in the efficiency with which they can make Sep15 and in how well they can use dietary Se *(82)*. Though the frequency of the T811/A1125 haplotype is 0.25 in Caucasians and 0.57 in African Americans, who have a higher incidence of prostate cancer *(80)*, no evidence of an effect of this polymorphism on prostate cancer risk has been reported nor found *(102)*. However, among African Americans (but not Caucasians), a difference in allele frequencies was seen in DNA from breast or head and neck tumours and that from cancer-free controls though the authors suggest that this difference is likely to be due largely to loss of heterozygosity at the *Sep15* locus *(80, 103)*.

The A1125 variant of Sep15 was found to be less responsive to the apoptotic and growth-inhibitory effects of Se than the G1125 variant *(104)*. In that study, the *Sep15* gene was shown to be down-regulated in 60% of malignant-mesothelioma cell lines and tumour specimens.

A Polish study of 325 lung cancer cases and 287 controls, all of whom were smokers, showed an effect of *Sep15* G/A1125 genotype that varied according to Se status *(105)*. Among individuals of lower Se status (below 50 μg/L), the risk was higher for those with the AA genotype compared to those with the GG genotype, whereas among those of higher Se status (above 50 μg/L), the opposite was the case. Though the effects on risk did not reach significance, there was a general significant association between Se concentration and lung cancer risk for the GG, GA and AA genotypes.

### 2.14.4. Selenoprotein P

A number of SNPs have also been identified in selenoprotein P (e.g. SEPP1, Ala234Thr, rs3877899), a selenoprotein believed to be involved both in protection from reactive oxygen and nitrogen species and in the transport of Se to tissues. Normally, the *SEPP1* gene is highly expressed in prostatic epithelium but it is down-regulated in a subset of human prostate tumours, mouse tumours and the androgen-dependent (LNCaP) and androgen-independent (PC-3) prostate cancer cell lines *(106)*. Our own results (*102*) from genotyping some 5,000 prostate cancer cases/controls from the population-based Prostate Cancer in Sweden (CAPS) study implicate SEPP1 in prostate cancer risk in a low-Se population.

Genetic variants at or near the SEPP1 locus may also be associated with advanced colorectal adenoma, a cancer precursor. Cases with a left-sided advanced adenoma ($n = 772$) and matched controls ($n = 777$), screen negative for polyps, were randomly selected from participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial *(107)*. Three variants in SEPP1, one of which was very rare, were significantly associated with advanced adenoma risk and a significant overall association with adenoma risk was observed for SEPP1 (global $p = 0.02$).

### 2.14.5. Thioredoxin Reductase 1

In the colorectal adenoma study described above *(107)* a significant 80% reduction for advanced colorectal adenoma risk was also seen for carriers of the variant allele at thioredoxin reductase 1 (TXNRD1) IVS1-181C>G (OR 0.20; 95% CI 0.07–0.55;

$p_{trend} = 0.004$). A significant overall association with adenoma risk for TXNRD1 (global $p = 0.008$) was observed.

### 2.14.6. Phospholipid Glutathione Peroxidase, GPx4

GPx4 decreases lipid hydroperoxide levels. A GPx4 C718T polymorphism (rs713041) which is known to be functional *(108–110)* has been shown to be linked to colorectal cancer risk in a pilot study *(111)*. Carriage of the T allele appears to be protective. Though no effect of this polymorphism has been found on risk of breast cancer, carriage of the T allele was found to be associated with mortality in 4,470 breast cancer cases *(112)*.

### 2.14.7. Summary of the Evidence on Selenoprotein Polymorphisms and Cancer Risk

It is difficult to draw clear conclusions from the evidence, as summarised in Tables 1 and 2, on selenoprotein polymorphisms and cancer risk. The situation appears to have become more confused as more studies are published. With regard to the GPx1 Pro198Leu polymorphism, some studies have shown an allele effect on GPx activity while others have not. Some authors have suggested that these disparities might be explained if the change in GPx activity were to be caused by another polymorphism that co-segregates with the studied polymorphism *(84)*. Interestingly, though only one study showed a significant effect of carriage of the Leu-allele on breast cancer risk (*84*), increased risk was observed in Leu homozygotes who were also homozygous for SOD2 16Ala (*93*), implying that the risk associated with this polymorphism is affected by other genotypes and perhaps other environmental factors as well. This is an illustration of the fact that cancer is a multifactorial complex disease and a combination of factors – not just a single polymorphism – is generally required to cause disease. It is also possible that some of the discrepancies noted above may relate to differing Se status between populations investigated. The fact that development of colorectal, breast and head and neck tumours is linked with loss of heterozygosity at GPx1 suggests that loss of GPx1 activity may increase cancer risk. Loss of heterozygosity in Sep 15 also occurs in breast or head and neck tumours, again suggesting that ability to make this selenoprotein may be important in reducing risk.

Though there are many fewer studies that have investigated associations between polymorphisms in thioredoxin reductase (TXNRD1 IVS1-181C>G) and selenoprotein P, significant effects on risk have been associated with these polymorphisms, i.e. risk of advanced colorectal adenoma.

As a general comment, however, genetic variants in other genes at or near the loci of the selenoprotein polymorphisms under study might co-segregate, contributing to the effects observed. All of the genetic variants that might contribute to such an effect must therefore be identified before disease risk can be definitely attributed to a particular polymorphism.

Table 1
GPx1: Proline/Leucine SNP at codon 198, 3p21. Effect of 198 Leu allele on cancer risk

| Cancer | No. Subjects | | SNP genotype *vs* Pro/Pro | OR (95% CI) * = significant | Location | Comments | Reference |
|---|---|---|---|---|---|---|---|
| | Cases | Contr | | | | | |
| Lung | 315 | 315 | Pro/Leu<br>Leu/Leu | **1.8 (1.2 -2.8)***<br>**2.3 (1.3 -3.8)*** | Finland | | RatnasingheD et al 2000 |
| Lung | 200 | 200 | Pro/Leu & Leu/Leu | 2.29 | Korea | Article in Korean, no CIs in abstract | Lee C et al. 2006 |
| Lung | 432 | 798 | Leu/Leu | 0.60 (0.35 -1.05) | Denmark | | Raaschou-Nielsen O et al. 2007 |
| Prostate | 82 | 123 | Pro/Leu<br>Leu/Leu | **0.38 (0.20-0.75)***<br>0.61 (0.27-1.40) | FYROM | Would expect stronger effect in Leu/Leu; small study | Arsova-Sarafinovska Z et al. 2008 |
| Prostate | 1433 | 780 | Pro/Leu<br>Leu/Leu | 1.07 (0.89-1.29)<br>0.93 (0.69-1.26) | Sweden | CAPS study | Rayman M et al. unpublished |
| Breast | 399 | 372 | Pro/Leu<br>Leu/Leu | 0.92 (0.68 -1.24)<br>0.77 (0.46 -1.27) | Canada | Pre-and post-menopausal | Knight J et al 2004 |
| Breast | 377 | 377 | Pro/Leu + Leu/Leu | **1.43 (1.07 -1.92)*** | Denmark | Postmenopausal | Ravn-Haren et al. 2006 |
| Breast | 1038 | 1088 | Pro/Leu<br>Leu/Leu | 1.10 (0.92 -1.32)<br>1.06 (0.79 -1.42) | US | Long Island Breast Cancer Study | Ahn J et al 2005 |
| Breast | 1229 | 1629 | Pro/Leu<br>Leu/Leu | 0.91 (0.77 -1.07)<br>1.07 (0.82 -1.40) | US | Nurses' Health Study | Cox D et al 2004 |
| Breast | 1262 | 1533 | Pro/Leu + SOD2 A/A<br>Leu/Leu +SOD2 A/A | 1.01 (0.83 -1.23)<br>**1.87 (1.09 -3.19)*** | US | Nurses' Health Study | Cox D et al 2006 |
| Bladder | 213 | 209 | Pro/Leu<br>+SOD2 V/A+A/A | **2.6 (1.5 -4.8)***<br>**6.3 (1.3 -31.2)*** | Japan | Pro/Leusignif. assoc. with advanced tumorstage | IchimuraY et al 2004 |

**Table 2**
**Effect of Selenoprotein SNPs on Cancer Risk**

| Selenoprotein SNP | Cancer | No. Subjects | | Comparison | OR (95% CI) | Location | Comments | Reference |
|---|---|---|---|---|---|---|---|---|
| | | Cases | Contr | | | | | |
| **GPx4**<br>C718T<br>rs713041 | Breast | 569 deaths | 3901 | T allele *vs* CC | **1.27 (1.13-1.43)* per T allele carried** | UK | Association of T allele with mortality in 4470 breast cancer cases | Udler M et al. 2007 |
| | Colorectal | 252 | 187 | CT+ TT *vs* CC | **0.60 (0.37-0.96)*** | UK | Carriage of T allele appears to be protective | Bermano G et al. 2007 |
| | Prostate | 1438 | 790 | CT+TT *vs* CC | 1.01 (0.91-1.32) | Sweden | CAPS study | Rayman M et al. unpublished |
| **Sep15**<br>G1125A linked C811T | Lung | 325 smokers | 287 smokers | GA *vs* GG<br>AA *vs* GG | 0.91 (0.64-1.32)<br>0.80 (0.39-1.65) | Poland | Above 80 µg/L Se, those carrying the G allele had increased risk | Jablonska E et al. 2008 |
| G1125A linked C811T | Prostate | 1419<br>318 | 781<br>**781** | CG/TA+TA/TA *vs* CG/CG | 1.03(0.86-1.24)<br>1.38 (1.05-1.83)* | Sweden | CAPS study<br>Men with PSA>100<br>Incr risk if TA homo/hererozygotes | Rayman M et al. unpublished |
| **TRR**<br>TXNRD1 IVS1-181C>G<br>rs35009941 | Advanced colorectal adenoma | 772 | 777 | GC+GG *vs* CC | **0.20 (0.07-0.55)*** | US | Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial<br>Carriage of G allele protective | Peters U et al. 2008 |
| **SEPP1**<br>rs3797310<br>rs2972994 -4166<br>rs3877899 | Advanced colorectal adenoma | 746<br>750<br>749<br>752 | 762<br>764<br>763<br>766 | AA *vs* GG<br>CT *vs* CC<br>CG *vs* CC<br>AG *vs* GG<br>AA *vs* GG | **1.53 (1.05-2.22)***<br>**0.73 (0.57-0.92)***<br>**P trend = 0.002***<br>0.99 (0.79-1.24)<br>0.98 (0.61-1.56) | US | Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial | Peters U et al. 2008 |
| rs3877899 | Prostate | 2975 | 2149 | AG *vs* GG<br>AA *vs* GG | 0.94 (0.82-1.07)<br>1.09 (0.83-1.42) | Sweden | CAPS study | Rayman et al. unpublished |

### 2.14.8. Hypermethylation of Promotor Regions Silence Selenoprotein Genes and Increase Cancer Risk

Abnormal DNA methylation patterns are associated with neoplasia and inactivation of tumour-suppressor genes. Hypermethylation of promoter regions of selenoprotein genes can effectively silence the gene resulting in loss (or reduced amount) of the selenoprotein and increasing cancer risk. Examples of an effect of promoter methylation on cancer risk are found for GPx3 in prostate cancer and Barrett's oesophagus and for the selenoprotein, methionine sulphoxide reductase in breast cancer metastasis.

Down-regulation of GPx3 occurs widely in prostate cancer. GPx3 was shown to be hypermethylated in LnCAP (lymph node metastasis) and PC3 (bone metastasis) prostate cancer cell lines and in 93% of primary prostate cancer tumours *(113)*. In a later study, the GPx3 promoter was found to be 90% methylated in prostate cancer tissue samples and its down-regulation was associated with higher rate of post-prostatectomy metastasis *(114)*. Somewhat decreased expression of GPx3 can be detected in morphologically benign prostate tissues adjacent to cancer tissue, implying that such a decrease precedes the development of frank malignancy. Deletions of GPx3 occurred in 39% of samples suggesting that deletion, as well as methylation, probably plays a major role in down-regulating the expression of this gene *(114)*. A reduction in mortality and tumour volume was observed when GPx3 was expressed in PC-3 xenograft mice suggesting that GPx3 has a tumour-suppressor role *(114)*.

GPx3 was also shown to be hypermethylated in 62% of Barrett's metaplasia, 82% of dysplasia and in 88% Barrett's adenoma samples *(115)*. Consistently reduced levels of GPx3 mRNA were seen in 91% of Barrett's adenoma samples. Monoallelic methylation was associated with partial loss of GPx3 expression in metaplasia while biallelic methylation and severe loss of GPx3 expression were most frequently seen in Barrett's adenoma samples ($p = 0.001$) (Table 3).

**Table 3**
**Frequency of GPx3 Promoter Hypermethylation According to Histomorphologic Diagnosis [Lee et al. *(115)*]**

| | *Histologic diagnosis* | | | |
|---|---|---|---|---|
| *GPx3 methylation status* | *Normal (n = 10)* | *Barrett's oesophagus (n = 21)* | *Barrett's dysplasia (n = 11)* | *Barrett's adenocarcinoma (n = 34)* |
| No methylation ($M^-$) | 10 (83%) | 8 (38%) | 8 (38%) | 4 (12%) |
| Monoallelic ($M^+/M^-$) | 2 (17%) | 7 (33%) | 8 (73%) | 14 (41%) |
| Biallelic ($M^+/M^+$) | 0 | 6 (29%) | 1 (9%) | 16 (47%)* |

M, methylation; ($^-$) negative; ($^+$) positive.
*Biallelic methylation was significantly more frequent in Barrett's adenocarcinoma ($P < 0.01$).

The last example of methylation affecting transcription of a selenoprotein relates to methionine sulfoxide reductase 1 (MsrB1), a selenoprotein linked with longer lifespan that is highly expressed in the weakly metastatic breast carcinoma cell line MCF7 but poorly expressed in the highly metastatic cell line MDA-MB231 *(116)*. The promoter of this selenoprotein is regulated by epigenetic modification as evidenced by the upregulation of the gene transcript after treatment with the demethylating agent, 5-aza-2′-deoxycytidine *(116)*. A CpG site within the Sp1 consensus site located 46 bp upstream of the transcription start site was found to be hypermethylated in the highly metastatic cell line suggesting a possible cause for the repression of transcription.

These examples clearly illustrate that functional *GPx3*, at least (and probably MsrB1), is important in reducing the risk of some cancers.

## 3. IN VITRO STUDIES AND ANIMAL EXPERIMENTS

Cell-culture studies have made a massive contribution to the understanding of the multiple mechanisms by which Se acts in the prevention of the incidence and spread of cancer. Such studies are too numerous to describe again here given that those that have made the most significant contribution to our current knowledge have already been covered under the appropriate mechanism sections.

There is similarly extensive experimental evidence from animal studies that indicates that Se supplementation reduces the incidence of cancer in animals, lowering the yield of tumours and reducing metastases *(25*, *59*, *62*, *63*, *76*, *117)*. It is difficult, however, to generalize from such studies to the human situation, as animal studies have generally used doses at least ten times greater than those required to prevent clinical signs of deficiency, which, on a per unit body-weight basis, are considerably higher than most human Se intakes. In recent years, evidence from transgenic models has been most useful in demonstrating that selenoproteins are important for the cancer-protective effects of selenium as in the studies quoted below.

A unique mouse model was developed by inter-breeding transgenic mice with reduced selenoprotein levels because of the expression of an altered selenocysteine-tRNA (i6A-) with mice that develop prostate cancer because of the targeted expression of the SV40 large T and small t oncogenes to that organ (C3 (1)/Tag) to give bigenic animals (i6A-/Tag) *(118*). The selenoprotein-deficient mice exhibited accelerated development of lesions associated with prostate cancer progression, suggesting that selenoproteins are important in reducing prostate cancer risk and development.

Transgenic mice with the same mutant selenocysteine-tRNA gene (i6A-) described above were fed selenium-deficient diets supplemented with 0, 0.1 or 2.0 μg Se (as selenite)/g diet *(119)*. Compared with wild-type mice, transgenic mice had more ($p <$ 0.05) azoxymethane-induced aberrant crypt formation (a preneoplastic lesion for colon cancer). Se supplementation significantly decreased the number of aberrant crypts and aberrant crypt foci in both wild-type and transgenic mice. It is clear from this study that a lack of selenoprotein activity causes increased colon cancer susceptibility but furthermore that low molecular weight selenocompounds were able to reduce preneoplastic lesions in these animals. This important study thus provided evidence that

*both* selenoproteins *and* low molecular weight selenocompounds are important for the cancer-protective effects of selenium (Irons et al. *(119)*).

## 4. EPIDEMIOLOGICAL STUDIES

Numerous cohort and nested case–control studies have shown that higher Se status is associated with a lower risk of malignancies or death from cancer (e.g. prostate, liver, lung, colon/rectum, oesophagus, gastric cardia, bladder, pancreas and thyroid) *(23*, *24*, *26*, *28*, *120–128)*.

Cancer mortality in relation to prediagnostic Se status was investigated in the large US Third National Health and Nutrition Examination Survey (NHNES III) *(120)*. Serum Se was measured at baseline in 13,887 adult participants who were then followed up for up to 12 years. Mean serum selenium concentration was 125.6 μg/L. The multivariate-adjusted hazard ratios (HR) comparing the highest (≥ 130.39 μg/L) with the lowest (< 117.31 μg/L) serum selenium tertile were 0.83 (95% confidence interval [CI] 0.72–0.96) for all-cause mortality and 0.69 (95% CI 0.53–0.90) for cancer mortality. The association between serum Se and all-cause and cancer mortality was nonlinear, showing an inverse association with total cancer mortality up to a serum Se concentration of around 130 μg/L (Fig. 4). While total cancer mortality began to rise again above a serum concentration of 150 μg/L, mortality from colorectal and prostate cancers continued to decline. Similar benefits of higher Se status on cancer mortality were seen in the smaller EVA study conducted among elderly French volunteers. There the risk of mortality from cancer was increased four-fold in volunteers in the bottom quartile of

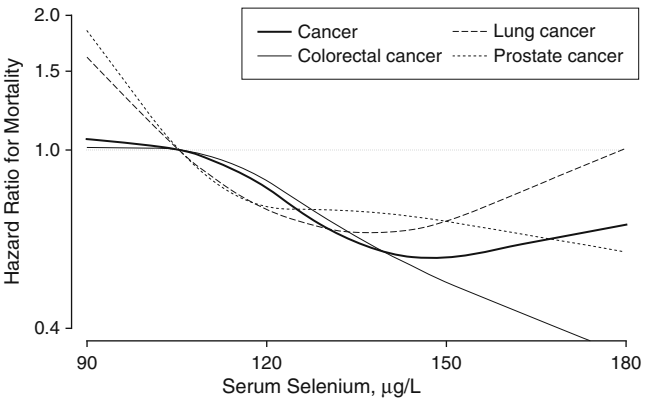


**Fig. 4.** Hazard ratios for all-cancer and colorectal, lung and prostate cancer mortality by serum Se concentration. The curves represent multivariate-adjusted hazard ratios based on restricted quadratic splines with knots at the 5th, 50th and 95th percentiles of the serum Se distribution. The reference value (hazard ratio, 1) was set at the 10th percentile of the serum Se level distribution (105.4 ng/ml). Hazard ratios were adjusted for age, sex, race/ethnicity, education, family income, menopausal status, cigarette smoking status, serum cotinine level, alcohol consumption, physical activity, body mass index and vitamin and/or mineral supplement use. The *p* values were 0.006 for all-cancer mortality, 0.72 for colorectal cancer mortality, 0.14 for lung cancer mortality and 0.77 for prostate cancer mortality. To convert Se to μmol/L, divide by 78.96. Reproduced with permission from reference *114*.

baseline plasma Se compared to those in the top quartile (relative risk, RR = 4.06; 95% CI 1.51–10.92, $p = 0.006$) *(129)*.

A few epidemiological studies have specifically investigated the association between Se and bladder cancer. Of seven prospective studies, five found a decreased risk of bladder cancer with high vs. low serum/toenail Se *(128)*. The largest of these prospective studies, carried out in the Netherlands, included 431 cases and 2,459 sub-cohort members. A significant inverse association between toenail Se concentrations and the risk of bladder cancer in the highest vs. lowest toenail Se quintile (RR 0.67; 95% CI 0.46–0.97) was reported *(121)*. The association was most pronounced in ex-smokers and in those with invasive transitional-cell carcinomas.

The relationship between baseline serum Se and the subsequent risk of death from oesophageal squamous cell carcinoma (ESCC) and gastric cardia cancer (GCC) was investigated over 15 years of follow-up of 1,103 subjects randomly selected from a larger trial cohort of all participants in the General Population Trial (the 5-year intervention did not involve Se) of Linxian, China. Compared with those in the lowest quartile of serum Se at baseline, those in the highest quartile had a 65% significant reduction in the risk of mortality from ESCC (RR: 0.35, 95% CI 0.16–0.81) and a 69% reduction in the risk of death from GCC (RR: 0.31, 95% CI 0.11–0.87) *(123)*.

The association between plasma Se concentration and subsequent risk of hepatocellular carcinoma (HCC) was investigated among chronic carriers of hepatitis-B and/or C virus in a cohort of 7,342 men in Taiwan (*24)*. Mean baseline Se concentration was significantly lower in the 69 men who developed HCC over 5.3 years of follow-up than in the 139 matched, healthy HBsAg-positive controls ($p = 0.01$). Adjusted odds ratios of HCC for subjects in increasing quintiles of plasma Se were 1.00, 0.52, 0.32, 0.19 and 0.62, respectively. The inverse association between plasma Se and HCC was most striking among cigarette smokers and among those with low plasma levels of retinol or various carotenoids.

Colorectal adenoma is closely associated with subsequent development of colorectal cancer. Peters and colleagues have tabulated observational studies of the effect of Se on colorectal tumours *(130)*. Such studies have generally supported a preventive role for selenium although not always with statistically significant effects. Jacobs and colleagues *(131)* carried out a pooled analysis of data from three studies that could be considered as prospective studies of Se and risk of colorectal adenoma. The Wheat Bran Fiber Trial, the Polyp Prevention Trial and the Polyp Prevention Study were 3–4-year interventions in subjects that had recently undergone adenoma removal, 1,763 of whom had baseline serum or plasma Se measured. None of the trials affected the risk of adenoma recurrence. Analysis of pooled data showed that those with baseline serum or plasma Se in the highest quartile (median 150 μg/L), when compared with those in the lowest quartile (median 113 μg/L), had significantly lower risk of adenoma recurrence (OR 0.66; 95% CI 0.50, 0.87). These results support previous findings that are suggestive of a beneficial effect of higher Se status on colorectal cancer risk.

In recent years, systematic reviews and meta-analyses have increasingly been used in an attempt to summarize the evidence for an effect of Se on the risk of some common cancers: a meta-analysis of 16 studies of Se and lung cancer showed that the summary relative risk (RR) for subjects with higher vs. lower Se exposures was 0.74 (95%

CI 0.57–0.97), suggesting a protective effect of Se against lung cancer *(124)*. Effects occurred primarily in populations with low-Se levels defined as serum Se < 100 mg/L or intake < 55 mg/d. Above a certain level, increasing Se intake had little further benefit, suggesting the existence of a threshold effect.

The strongest evidence for an effect of Se on a specific cancer site relates to its effect on prostate cancer. Large prospective studies of Se in prostate cancer are summarized in Table 4 *(21*, *23*, *26*, *28*, *125*, *132–135)*. Most interestingly, many of these studies have examined the effect of Se separately on advanced prostate cancer (cancer that has spread beyond the prostate) and found the effect to be greater than on localized prostate cancer *(23*, *26*, *28*, *125)*. Recent systematic reviews and meta-analyses have reinforced these findings. A systematic review and meta-analysis of 16 studies *(126)* found that the pooled relative risk of prostate cancer for any intake of Se (average between first and fourth quintile or first and third quartile, compared to the lowest

**Table 4**
**Large Prospective Studies of Prostate Cancer/Advanced Prostate Cancer Using Tissue Indicators of Exposure**

| Study | Population | No. of cases | Indicator of exposure | Comparison High *vs.* low | RR[1] | 95% confidence interval | P for trend |
|---|---|---|---|---|---|---|---|
| Knekt *et al.* 1990 | Finland General population | 51 | Serum | Quintile | 1.15 | – | 0.71 |
| Yoshizawa *et al.* 1998 | USA Health professionals | 181 | Toenails | Quintile | 0.35[2] | 0.16–0.78* | 0.03 |
| Nomura *et al.* 2000 | USA Hawaii Japanese ancestry | 249 | Serum | Quartile | 0.5 | 0.3–0.9* | 0.02 |
| | Non smoker | 87 | | | 0.8 | 0.4–1.9 | 0.93 |
| | Ex-smoker | 86 | | | 0.5 | 0.2–1.1 | 0.03 |
| | Current smoker | 76 | | | 0.2 | 0.1–0.8 | 0.02 |
| | Localised disease | 120 | | | 0.8 | 0.4–1.8 | 0.76 |
| | Advanced disease | 64 | | | 0.3[2] | 0.1–0.8 | 0.01 |
| Helzlsouer *et al.* 2000 | USA Washington County | 117 | Toenails | Quintile | 0.58 | 0.29–1.18 | 0.27 |
| | | | | | 0.38[3] | 0.17–0.85* | 0.12 |
| Goodman *et al.* 2001 | USA CARET Trial asbestos workers/ current/ex-smokers | 235 | Serum | Quartile | 1.02 | 0.7–1.6 | 0.69 |
| | retinol/β-carotene arm | 111 | | | 0.75 | 0.41–1.36 | 0.40 |
| | placebo arm | 124 | | | 1.52 | 0.78–2.79 | 0.12 |
| Brooks *et al.* 2001 | USA Baltimore | 52 | Plasma | Quartile | 0.24 | 0.08–0.77* | 0.01 |

**Table 4**
**(Continued)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| van den Brandt *et al*. 2003 | Netherlands Cohort Study | 540 | Toenails | Quintile | 0.69 | 0.48–0.99* | 0.008 |
| | Never smoker | 72 | | | 1.19 | 0.48–2.92 | |
| | Ex-smoker | 300 | | | 0.46 | 0.27–0.79* | |
| | Current smoker | 168 | | | 0.97 | 0.42–2.22 | |
| | Localised disease | 189 | | | 0.72 | 0.42–1.24 | 0.043 |
| | Advanced disease | 183 | | | 0.62[2] | 0.37–1.05 | 0.020 |
| Li *et al.* 2004 | USA Physicians' Health Study | 586 | Plasma | Quintile | 0.78 | 0.54–1.13 | 0.16 |
| | Baseline PSA>4 | 228 | | | 0.49 | 0.28–0.86* | 0.002 |
| | Baseline PSA<4 | 293 | | | 0.77 | 0.48–1.22 | 0.59 |
| | Localised disease | 348 | | | 0.97 | 0.64–1.49 | 0.91 |
| | Advanced disease | 171 | | | 0.52[2] | 0.28–0.98* | <0.05 |
| Peters *et al.* 2006 | USA PLCO Trial | 724 | Serum | Quartile | 0.84 | 0.62–1.14 | 0.70 |
| | High vitamin E intake (≥28 IU/d) | 363 | | | 0.58 | 0.37–0.90* | 0.05 |
| | Multi-vitamin use | 302 | | | 0.61 | 0.36–1.04 | 0.06 |

*denotes statistically-significant effect

[1]RR relative risk for highest versus lowest category

[2]Advanced disease

[3]Adjusted for BMI at age 21, education and hours since last meal

intake category) was 0.72 (95% CI 0.61–0.84) for cohort studies and 0.74 (0.61–1.39) for case–control studies. Sub-group analysis showed a stronger protective effect among those with advanced disease with a dose–response trend, though the results were not significant, probably because of small sample size. Brinkman and colleagues *(128)* carried out a meta-analysis of 20 studies in which the pooled standardized mean difference between serum/plasma/toenail Se in cases and controls was −0.23 (−0.40, −0.05; $p = 0.01$) indicating an inverse association between Se status and risk of prostate cancer.

The recent World Cancer Research Report *(136)* which systematically reviewed the available research on diet and cancer prevention concurred with the above findings, concluding that there is strong evidence from trials and cohort studies that Se probably protects against prostate cancer and limited evidence that it protects against lung, colorectal and stomach cancers.

## 5. INTERVENTION STUDIES

A Cochrane review of randomised trials comparing antioxidant supplements with placebo for prevention of gastrointestinal cancers *(137)* found that Se significantly decreased the risk (RR 0.49, 95% CI 0.36–0.67). Notably, Se administered vs. placebo

to high-risk groups (i.e. carriers of hepatitis-B surface antigen and members of families with high incidence of liver cancer) for 2–4 years reduced the incidence of hepatocellular carcinoma by 50% (RR 0.50, 95% CI 0.35–0.71). The Bjelakovic Cochrane review concludes "Se alone (of antioxidant supplements) may have preventive effects on cancer. This finding, however, is based on trials with flaws in their design and needs confirmation in properly conducted randomised clinical trials."

Bardia and colleagues systematically reviewed the trial evidence for the effect of Se in primary cancer prevention *(138)*. Se gave a pooled relative risk (RR) for cancer incidence of 0.88 (95% CI 0.77–1.00) from pooling four trials with considerable heterogeneity ($I^2 = 52\%$). When the two trials that assessed Se in combination with beta-carotene and vitamin E were excluded, heterogeneity disappeared ($I^2 = 0\%$) and benefit increased: for incidence RR 0.69 (95% CI 0.56–0.85) and death RR 0.61 (95% CI 0.42–0.89). The results of the Nutritional Prevention of Cancer (NPC) trial were the major driver of the benefits found in that analysis.

The NPC trial provided compelling evidence that Se can reduce cancer incidence and mortality *(139)*. Of people with a history of non-melanoma skin cancer, 1,312 were randomised to 200 mcg Se/day (as high-Se yeast) vs. yeast placebo. After 4½ years of treatment and 6½ of follow-up, there was no effect on the primary end point of skin cancer but those receiving Se had 50% lower total cancer mortality (RR 0.50; 95% CI 0.31–0.80) and 63% lower cancer incidence (RR 0.63; 95% CI 0.47–0.85) with fewer cancers of the prostate (RR 0.37; 95% CI 0.18–0.71), colon/rectum (RR 0.42; 95% CI 0.18–0.95) and lung (RR 0.54; 95% CI 0.30–0.98). No effect on breast cancer was seen but there were only 332 women.

In follow-up analyses, though the effects of Se were attenuated, they remained significant for total cancer incidence (Cox proportional hazards model, HR 0.75; 95% CI 0.58–0.97) and prostate cancer incidence (HR 0.48; 95% CI 0.28–0.80) *(140)* but just missed significance for colorectal cancer incidence (HR 0.46; 95% CI 0.21–1.02; $p = 0.057$). Analysis by initial Se status showed that the strongest effect of Se was seen in those who fell into the bottom tertile (<105 μg/L) of plasma Se at baseline: HR total cancer 0.51, 95% CI 0.32–0.81 *(140)*; HR lung cancer 0.42, 95% CI 0.18–0.96; odds ratio (OR) prevalent colorectal adenoma 0.27, 95% CI 0.09–0.77 *(141, 142)*. Furthermore, when adjustment was made for the lower rate of PSA measurement in the Se-treated group, only those men with baseline plasma selenium <106 μg/L still showed a lower risk of prostate cancer on Se supplementation. By contrast, participants in the highest tertile (>122 μg/L) had a non-significant elevated incidence of total cancer incidence (HR 1.20, 95% CI 0.77–1.86) *(140)*.

Although follow-up analyses confirmed initial findings that Se supplementation was not statistically significantly associated with the incidence of basal-cell carcinoma (HR 1.09; 95% CI 0.94–1.26), the extended treatment period raised the elevated risk of squamous cell carcinoma and total non-melanoma skin cancer to statistically significant levels (HR 1.25; 95% CI 1.03–1.51 and HR 1.17; 95% CI 1.02–1.34, respectively) *(143)*. However, it must be remembered that the subjects in the NPC Trial were all skin cancer patients whose skin had sustained heavy sun-damage *(143)*. In fact when subjects were divided into tertiles according to baseline Se status, those in the bottom tertile, whose

status resembled that found in Europe, did not have an increased risk of squamous cell carcinoma (HR 0.87; 95% CI 0.62, 1.22). Of more concern is the fact that more recent analysis of NPC trial data showed an increased risk of self-reported type-2 diabetes in those supplemented with Se, though the effect was significant only in those in the top tertile of plasma Se at baseline *(144)*.

Overall, the trial showed that the protective effect of selenium was confined to males (HR 0.67; 95% CI 0.50–0.89) and was most pronounced in former smokers and in those in the bottom tertile of plasma Se at baseline *(140)*. It is notable that most of the population of Europe would fall into the bottom tertile of plasma Se, as defined in that study.

The later Selenium and Vitamin E Cancer Prevention Trial (SELECT) that investigated the effect of Se and vitamin E on prostate cancer risk showed that giving 200 μg Se/d (as selenomethionine) to a population of men of mean plasma Se 136 μg/L did not reduce the risk of localized prostate cancer *(145)*. In the light of NPC trial results, it is unfortunate that SELECT had no subjects within the range of Se status (i.e. <106 μg/L) that had previously shown benefit from Se supplementation on prostate cancer risk. In SELECT men, baseline serum Se ranged from 122.9 to 150.0 μg/L, which in fact, according to the NPC trial, put them into the category of non-significant increased risk from Se supplementation: in fact there were some potential indications of toxic effects in terms of alopecia and grades 1–2 dermatitis in the Se group.

Furthermore, SELECT supplemented with SeMet rather than Se-yeast as used in the NPC trial. As Se-yeast contains only some 60–70% of its total Se as SeMet *(146)*, it is possible that some other Se species than SeMet may have been responsible for the beneficial effects seen in the NPC trial.

Unfortunately SELECT results tell us nothing about the effect of Se on risk of advanced disease on which a number of studies have suggested a greater effect *(9, 102)* only 1.1% of cases were non-localised – nor on men of lower Se status. Clearly while at least one third of NPC men did not have optimal selenoprotein P or even GPx concentration/activity, this was not true of SELECT men, all of whose selenoproteins were likely to have been optimised *(147)*. Therefore if selenoproteins are important in cancer prevention, no effect would have been seen in SELECT, as was indeed the case. Such a trial conducted in Europe where Se status is substantially lower might have shown a very different outcome.

## 6. INTERVENTION WITH SELENIUM IN CANCER PATIENTS

A number of randomized controlled trials have been initiated in men with localized prostate cancer to see if supplementation with high-Se yeast can inhibit spread beyond the prostate. Thus the Watchful Waiting Trial *(148)* is being conducted in the Arizona Cancer Center in men with prostate cancer who have chosen watchful waiting rather than active intervention while my colleagues and I are conducting a double-blind, placebo-controlled, randomised phase II trial of Se supplementation in men with localised prostate cancer in men under active surveillance for prostate cancer at the Institute of Cancer Research, UK.

A few clinical trials have used Se supplementation in combination with DNA-damaging chemotherapeutic agents such as cisplatin, doxorubicin and irinotecan *(149)*.

These agents produce bulky DNA-adducts that are repaired by nucleotide excision repair (NER) of DNA. Se appears to be able to enhance DNA repair but only in cells with normal p53 *(13)*. Se supplementation elevates the expression of proteins responsible for recognition of DNA damage, selectively protecting genetically normal cells from DNA-damaging chemotherapeutic agents, while simultaneously offering no detectable protection to cells either completely lacking p53 or possessing only mutant p53 *(150)*. It is important to note that as many as 70% of tumours have a mutant p53 phenotype. Thus Se appears to be able to protect tissues from dose-limiting toxicity, reducing DNA damage, the frequency of chromosomal aberrations, the number of aberrant metaphases and the frequency of apoptotic cells and allowing delivery of higher chemotherapeutic doses without affording protection to cancer cells *(150, 151)*. Other mechanisms have also been implicated in the sensitization of cancer cells to chemotherapeutic agents by Se *(152, 153)*.

## 7. TISSUE SPECIFICITY AND TOTALITY OF THE EVIDENCE

Evidence from molecular biology (selenoprotein SNPs and methylation of promoter regions of selenoprotein genes) suggests that adequate Se intake may be able to affect favourably the risk of prostate, lung and bladder cancers, and oesophageal and colorectal adenomas.

Aside from that evidence, while it is safe to draw conclusions from well-run randomised controlled trials, the evidence from observational or case–control studies, even when nested within a prospective cohort study, is subject to some uncertainty owing to the effect of inflammation (or acute phase response) on blood/plasma/serum (or perhaps even toenail) Se concentration *(154, 155)*. The plasma concentration of Se decreases in proportion to the magnitude of the inflammatory response. Plasma selenoprotein concentration declines, most notably that of selenoprotein P, with inflammatory activity *(154, 156)*. Even the early stages of degenerative disease and cancer may have a subclinical inflammatory component *(154, 157, 158)*. Thus chronic low-level inflammation may long precede the appearance of symptoms. For instance, increasing age, smoking, symptoms of chronic bronchitis, Helicobacter pylori and Chlamydia pneumonia infections and body mass index were all associated with raised concentrations of C reactive protein, a recognised marker of inflammation, in UK men aged 50–69 years from general practice registers *(157)*. Hence when interpreting plasma selenium concentrations, a marker of the inflammatory response, such as CRP, should always be included to distinguish true nutritional depletion from the inherent effects of disease *(154)*.

Given the caveats referred to above, and therefore summarizing only the results found from trial evidence, for individual cancers the following conclusions can be drawn:

- Prostate cancer: There is evidence that Se probably protects against prostate cancer in men of relatively low-Se status (baseline plasma Se < 106 μg/L) *(140)*, but not in men of replete Se status (i.e. serum Se >123 μg/L) *(145)*. Se seems to have a specific role in down-regulation of the androgen receptor which may be a key factor in its effect.
- Liver cancer: There is a likely beneficial effect of Se on the risk of hepatocellular carcinoma in high-risk groups (i.e. carriers of hepatitis-B surface-antigen and members of families with high incidence of liver cancer). However, the relevant trials have flaws in

their design and the work needs confirmation in properly conducted randomised clinical trials *(137)*.
- Lung cancer: There is a trend towards a reduction in risk of incident lung cancer with Se supplementation though a significant reduction in risk occurred only in those with a plasma Se concentration less than 106 μg/L.
- Colorectal cancer and its precursor, colorectal adenoma: Se supplementation is associated with a trend towards a reduction in risk of incident colorectal cancer and a significant decrease in prevalent adenomatous polyps, particularly in current smokers and subjects with baseline plasma selenium <105.5 μg/L *(140–142)*.
- Squamous cell carcinoma: Se supplementation was associated with a higher risk of SCC in participants in the highest two tertiles of baseline plasma selenium, i.e. >105.6 μg/L though there was no increased risk in participants whose plasma Se was below 105.2 μg/L *(143)*.

In general, trial evidence for a protective effect of Se, which comes mainly from one study, relates only to men, is most pronounced in former smokers and in those with plasma Se <105 μg/L *(140)*. Indeed, there is a suggestion that those with plasma Se above 122 μg/L may have an elevated incidence of total cancer with selenium supplementation *(140)*. It is notable that data from the Third National Health and Nutrition Examination Survey (NHANES III) show that around half of the US population fall into this category and should probably *not* be exposed to additional Se *(159)*. On the other hand, plasma Se concentrations in Europe, New Zealand and many parts of China lie below 105 μg/L, and those populations might benefit from an increased Se intake to reduce cancer risk.

## 8. FUTURE RESEARCH DIRECTIONS

From the arguments presented above that show that chronic low-grade inflammation can effect measures of Se status long before the appearance of disease, it is clear that only intervention studies can give a truly reliable picture of the influence of Se on disease risk and so we need more trials, though it should be noted that these need to be in appropriate populations, i.e. those of low-Se status or in specific high-risk genotype groups. Unfortunately randomised controlled trials (RCTs) are extremely expensive.

Recent studies have implicated high serum Se or Se supplementation in increased risk of type-2 diabetes *(144*, *160*, *161)*. Furthermore, it appears that high serum Se concentrations may be associated with high total cholesterol, LDL-cholesterol, triglycerides, apo B and apo A1 levels *(162*, *163)*. We must therefore work towards identifying a safe intake of Se which, while reducing cancer risk, will not increase the risk of other serious conditions.

Given these and other considerations, we badly need a large RCT of the effect of Se on all cancers which fulfils the following criteria:

- carried out in a population with a relatively low baseline Se intake, such as a European population;
- includes enough women to look at the effect on risk in females as there has been no sizeable trial of Se supplementation in women;

– uses a lower dose of Se, e.g. 100 μg/d, as if such a dose is effective in reducing cancer risk, it may avoid adverse effects that may appear at a higher daily dose. Furthermore, it will be easier to implement a public-health policy to achieve that level of intake.

Trials with *Se*-methylselenocysteine, particularly in early prostate cancer, are also needed and will no doubt follow on from the currently planned pharmacokinetic studies with this small molecular weight Se compound.

Most importantly, given the data summarised above that has appeared in the last 10 years showing that selenoprotein genotype can affect cancer risk, trials in subjects of known SNP genotype for selenoproteins (or genes in related pathways) must be initiated as we can no longer assume that each person's Se requirement to reduce cancer risk is the same.

Aside from trials, adjunctive therapy with Se in cancer chemotherapy has clear potential for a better prognosis from treatment as outlined above.

## 9. CONCLUSIONS AND RECOMMENDATIONS FOR INTAKE/DIETARY CHANGES

Given what we know about the effect of selenoprotein genotype on the ability to make selenoproteins and on cancer risk, there will be considerable individual variation in Se requirements for optimal health. To date, few epidemiological studies have taken genotype into account and so the recommendations that follow cannot be prescriptive for an individual.

From prospective studies, the mean/median level of plasma Se required for a significant reduction in cancer risk ranges from > 84 μg/L (e.g. for oesophageal and gastric cardia cancer in China) to 147 μg/L (e.g. for prostate cancer in Hawaii) according to the study, while from trial data, the minimum mean plasma Se for significant reduction in cancer risk in an Eastern US population in the NPC Trial ranged from 105 μg/L (all cancers) to 123 μg/L (prostate cancer) *(164)*. The minimum Se intake required to achieve these plasma concentrations ranges from just below the US RDA/UK RNI (55–75 μg/d) level to a total intake of around 140 μg/d from dietary Se [or Se-yeast, which is similarly absorbed and retained *(146)*. This assertion is based on results of a UK supplementation study in healthy volunteers with a baseline dietary intake of approximately 40 μg/d in which a further 100 μg Se/d as Se-enriched yeast raised plasma Se from 90.3 to 148.4 μg/L *(165)*. To date, no cancer trial has used a level of dose that would give a total intake of 140 μg Se/d as suggested above, all having opted for an additional 200 μg Se/d or more.

Trial evidence, which as explained above is more reliable, has only supplied information on a supplemental dose of 200 μg Se/d. While this appeared to benefit men of relatively low-Se status (plasma Se $\leq$ 105 μg/L), there was no benefit on the risk of prostate cancer for men of replete Se status (serum Se >123 μg/L) *(145)*. Those with plasma Se >122 μg/L, if given 200 μg Se/d, may run the risk of developing squamous cell carcinoma *(143)* and type-2 diabetes *(144)*. The advisability of supplementing individuals of already-replete status (say 125 μg/L or more) *(147)* with Se must be questioned. Certainly it should be apparent that in populations that already have a mean

baseline intake at the level associated with reduced cancer risk, e.g. the Prostate, Lung, Colorectal and Ovarian Cancer Trial population, where mean plasma Se was 141.3 μg/L *(135)*, or SELECT, where mean serum Se was 136 μg/L, no significant benefit at higher intake/status should be expected, nor indeed was seen in the higher Se status individuals in those populations. Such populations should *not* be exposed to additional dietary Se or supplementation.

Additional Se may benefit those living in regions of low-to-moderate Se status (plasma Se $\leq$ 105 μg/L). Good food sources of Se in such regions are scarce but include the following:-

Brazil nuts, kidney, fish, liver and shellfish *(164)*. However, many people rarely eat such foods. Men in this category (e.g. Europeans), particularly if there is a family history of prostate cancer, should be able to take an additional daily dose of up to 200 μg Se/d without adverse effects. There appears to be no case for women of low-to-moderate Se status supplementing with a dose higher than 100 μg Se/d, as 100 μg/d should be able to raise plasma Se to near 150 μg/L, a level associated with decreased risk in a number of studies *(164)*.

Definitive guidelines on optimal intake await further research and, for individuals rather than populations, these must ultimately be genotype related.

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